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# Lack of digalactosyldiacylglycerol increases the sensitivity of *Synechocystis* sp. PCC 6803 to high light stress

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ABSTRACT

**The physiological role of digalactosyldiacylglycerol (DGDG) in photosynthesis was examined using a *dgdA* mutant of *Synechocystis* sp. PCC 6803 that is defective in the biosynthesis of DGDG. The *dgdA* mutant cells showed normal growth under low light (LL) conditions. However, their growth was retarded under high light (HL) conditions and under $Ca^{2+}$- and/or $Cl^-$-limited conditions compared to wild-type cells. The retardation in growth of the mutant cells was recovered by exogenous supply of DGDG in the growth medium. The *dgdA* mutant showed increased sensitivity to photoinhibition. Although both photodamage and repair processes of photosynthesis were affected, the repair process was more severely affected than the photodamage process, suggesting that DGDG plays an important role in the photosynthetic repair cycle.**


## 1. Introduction

In cyanobacteria, algae, and plants, thylakoid membranes are the site of the photochemical reactions of oxygenic photosynthesis, which are performed by two protein–pigment complexes, photosystem II (PSII) and photosystem I (PSI) [1]. The lipid composition of thylakoid membranes is highly conserved among oxygenic photosynthetic organisms and is composed of uncharged lipids including monogalactosyldiacylglycerol (MGDG; 40–50% of total lipids) and digalactosyldiacylglycerol (DGDG; 20–30%), as well as anionic lipids including sulfoquinovosyldiacylglycerol (SQDG; 20–30%) and phosphatidylglycerol (PG; 5–15%) [2]. The majority (80–90%) of lipids in thylakoid membranes are glycolipids, namely, MGDG, DGDG, and SQDG, in contrast to other biological membranes in which phospholipids are the major lipid components. Recent X-ray crystallographic analysis of PSII [3,4] and PSI [5] complexes from *Thermosynechococcus elongatus* revealed the presence of 14 (six molecules of MGDG, four molecules of DGDG, three molecules of SQDG, and one molecule of PG) and four (one molecule of MGDG and three molecules of PG) lipid molecules per PSII and PSI monomer, respectively. Therefore, it is thought that lipid molecules might play important roles not only in the formation of the lipid-bilayer, but also in the construction of the PSI and PSII complexes. However, the specific photosynthetic function of each lipid class is not fully understood. In the present study, we focused on the physiological role of DGDG, a component of PSII.

The physiological importance of DGDG in photosynthesis has been studied using DGDG-deficient mutants of *Arabidopsis thaliana* [6–8] and *Synechocystis* sp. PCC 6803 [9,10]. In *Arabidopsis*, two genes, *DGD1* and *DGD2*, are involved in the biosynthesis of DGDG. The growth and photosynthetic activity were severely affected in a *dgd1* mutant in which the *DGD1* gene was disrupted and the amount of DGDG was reduced to 1% of total lipids [6]. A *dgd1 dgd2* mutant, in which both the *DGD1* and *DGD2* genes were disrupted and only a trace amount of DGDG was detected, had a more severe phenotypic disruption than the *dgd1* mutant [8]. These results suggest that DGDG is crucial for plant development and photosynthesis in *Arabidopsis*. Recently, our group and Awai et al. identified a *dgdA* gene presumably encoding a DGDG synthase of *Synechocystis* sp. PCC 6803 [9,10]. Since this disruption mutant of *dgdA* contained no detectable levels of DGDG [9,10], it was considered to be a good tool to elucidate the role of DGDG in photosynthesis. Analyses of the mutant in our previous studies demonstrated an important role of DGDG on the donor side of PSII through the binding of extrinsic proteins required for stabilization of the oxygen-evolving complex [10]. Requirements for DGDG on the donor side of PSII have also been elucidated by analysis of the *dgd1 dgd2 Arabidopsis* mutant using flash-induced chlorophyll

*Abbreviations:* DGDG, digalactosyldiacylglycerol; HL, high light; LL, low light; MGDG, monogalactosyldiacylglycerol; SQDG, sulfoquinovosyldiacylglycerol; PG, phosphatidylglycerol; PSI, photosystem I; PSII, photosystem II

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fluorescence [11]. However, since the *dgdA* mutant showed normal photoautotrophic growth [9,10] despite the impairment on the donor side of PSII, the physiological role of DGDG in *Synechocystis* remains obscure.

In this study, we examined the effects of high light (HL) stress or nutrient deficiency on the growth and photosynthetic activity of the *dgdA* mutant. We found that HL stress or $CaCl_2$-depletion from the growth medium significantly affected the growth of *dgdA* mutant cells. Further analysis of the *dgdA* mutant demonstrated that the acceleration of damage to photosynthesis under HL and inhibition of recovery of photodamaged photosynthesis leading to the retardation of growth under HL conditions occurs simultaneously as a result of a deficiency of DGDG.

## 2. Materials and methods

### 2.1. Organisms and growth conditions

A disruption mutant of the *dgdA* gene (*slr1508*) of *Synechocystis* sp. PCC 6803 constructed previously [10] is referred to as the *dgdA* mutant. The wild-type and the *dgdA* mutant of *Synechocystis* sp. PCC 6803 were grown photoautotrophically at 25 or 30 °C in BG-11 medium under continuous fluorescent white light at an intensity of 20 or 200 μmol photons $m^{-2}$ $s^{-1}$. In the case of the *dgdA* mutant, 20 μg/ml kanamycin was added to the growth medium. The growth of the cells was monitored by assessing the optical density at 730 nm ($OD_{730}$).

### 2.2. Analysis of photosynthetic activity

Photosynthetic oxygen-evolving activity of intact cells was measured by a Clark-type oxygen electrode according to Gombos et al. [12]. The samples were illuminated with white light filtered through thermo-cutting and red filters. Chlorophyll concentrations were determined by the method of Arnon et al. [13].

### 2.3. Purification of lipids

Total lipids were extracted from wild-type cells of *Synechocystis* sp. PCC 6803 by the method of Bligh and Dyer [14]. Lipids were separated by thin layer chromatography, as described previously [15]. Each lipid class extracted from the thin layer chromatography plates was dissolved in ethanol as a stock solution at a concentration of 2 mM. When the growth medium was supplemented with lipid, the stock solution was added to growth medium at a final concentration of 10 μM. The amount of lipid was quantified by gas chromatography, as described previously [15].

### 2.4. Photoinhibition and recovery of photosynthesis

To assay the susceptibility of *dgdA* mutant cells to HL, wild-type and mutant cells that had been suspended with BG-11 medium to 10 μg Chl/ml were illuminated at 25 °C with white light at 2500 μmol photons $m^{-2}$ $s^{-1}$ in the presence or absence of 0.1 mg/ml lincomycin. Recovery of photosynthetic activity after photoinhibition was measured in cells that had been subjected to photoinhibitory treatment at 30 °C for 60 min in the presence of lincomycin, washed twice with fresh BG-11 to remove the lincomycin, and then allowed to recover oxygen-evolving activity under low light (LL) (20 μmol photons $m^{-2}$ $s^{-1}$) at 25 °C.

## 3. Results

### 3.1. Growth of the dgdA mutant under HL or $Ca^{2+}$- and/or $Cl^{-}$-limited conditions

To examine the effects of DGDG deficiency on photosynthesis in vivo, the photoautotrophic growth of *dgdA* mutant cells was compared to that of wild-type cells under different conditions (Figs. 1 and 2). Fig. 1 shows the effects of illumination at LL (20 μmol photons $m^{-2}$ $s^{-1}$) and HL (200 μmol photons $m^{-2}$ $s^{-1}$) on the growth at 25 or 30 °C. Under LL conditions, the mutant cells grew at almost the same rate as the wild-type cells at both 25 °C (Fig. 1A) and 30 °C (Fig. 1B). Under HL conditions, however, the mutant cells grew significantly more slowly than the wild-type cells (Fig. 1C and D). After cultivation for 150 h, the mutant cells exhibited photobleaching and stopped growing, while the wild-type cells continued to grow. Such growth retardation was more apparent at 25 °C than at 30 °C. In contrast, when cells were cultured in the presence of DGDG, the mutant cells showed normal growth comparable to the wild-type even under HL conditions. Lipid analysis of the wild-type and mutant cells cultured with DGDG showed that the mutant cells contained 2.2% of DGDG in total lipids, while the wild-type cells contained 15% of DGDG and the lipid composition of the wild-type cells was not changed by cultivation with DGDG (data not shown). These results indicate that DGDG was incorporated into the mutant cells and it restored the growth of mutant cells under HL conditions although the level of DGDG was lower than that of DGDG in wild-type cells. It is, therefore, suggested that DGDG is required for the optimal growth of

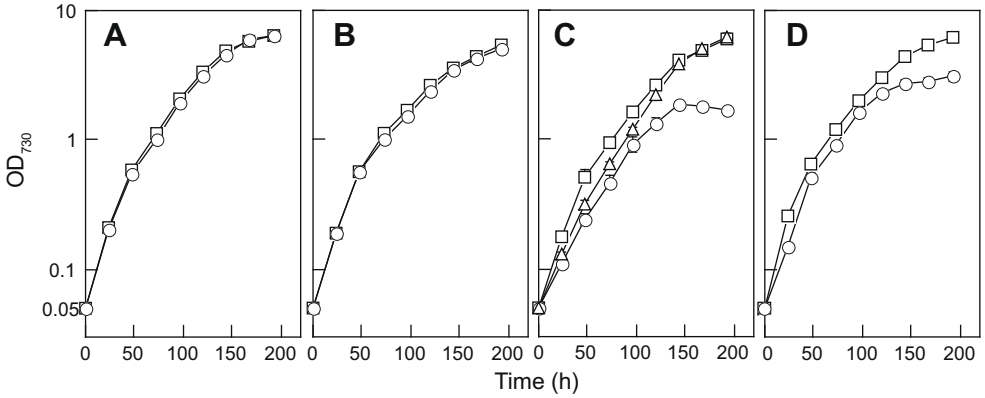


**Fig. 1.** Effects of low light (LL) and high light (HL) illumination on photoautotrophic growth of wild-type and *dgdA* mutant cells. Cells were cultured under LL (20 μmol photons $m^{-2}$ $s^{-1}$; A and B) or HL (200 μmol photons $m^{-2}$ $s^{-1}$; C and D) conditions at 25 °C (A and C) or 30 °C (B and D). Growth of wild-type (□) and *dgdA* mutant (○) cells was monitored by optical density at 730 nm. Growth of *dgdA* mutant cells in the presence of 10 μM DGDG was also monitored under HL conditions at 25 °C (△ in C). Error bars represent S.D. based on mean values of three independent cultures even though they are not clearly seen in the figure because of low deviation.